

iGEM 2016 – SDU	
Title: iTRAQ labelling	Date issued: 2016.10.04
SOP number: SOP0040	Review date: 2016.10.11
Version number: 01	Written by: Joel Vej-Nielsen

1. Purpose

Tagging proteins for MS/MS analysis.

2. Area of application

This procedure is valid for digested protein samples.

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
SpeedyVac			
Vortex			
Centrifuge			

4. Materials and reagents – their shelf life and risk labelling

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
iTRAQ Reagent 114				
iTRAQ Reagent 115				
iTRAQ Reagent 116				
iTRAQ Reagent 117				
ethanol				
Low binding eppendorf tubes				

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0037 - iTRAQ sample preparation

SOP0038 – Qubit® Protein Assay Kits

SOP0039 – C8 and C16 column purification

SOP0041 - TiO₂ purification

7. Environmental conditions required

8. Procedure

- 8.1 Allow iTRAQ™ reagent vials to reach room temperature.
- 8.2 Spin to collect solutions in the bottom of the tubes.
- 8.3 Add 70 µl of ethanol to each iTRAQ™ reagent vial
- 8.4 Vortex each vial and then spin briefly
- 8.5 Transfer the contents of one iTRAQ™ reagent vial to each sample tube containing 100µl of protein.
- 8.6 Vortex each tube and then spin briefly
- 8.7 Incubate the tubes for 1 hour at room temperature
- 8.8 Quench labelling with chemicals
- 8.9 Pool 5 µl of each sample and analyze on mass spectrometer
- 8.10 If the results are good pool the remaining amount of proteins.
- 8.11 Dry on vacuum centrifuge

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks

10. Time consumption

- Total-time 2h
- Hands-on-time 1h

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.10.04 / JVN	01	The SOP has been written
16.10.13 / JR		The SOP has been reviewed

12. Appendixes